Pharmacokinetics of S-ketamine and R-ketamine and their active metabolites after racemic ketamine or S-ketamine intravenous administration in dogs sedated with medetomidine

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Abstract: Objective: To assess the differences in the pharmacokinetic profiles of S-ketamine, R-ketamine and their metabolites, S-norketamine and R-norketamine, and to measure relevant physiologic variables after intravenous administration of racemic (RS) ketamine or S-ketamine alone in Beagle dogs sedated with medetomidine. Study design: Experimental, blinded and randomized crossover study. Animals: A total of six (three female and three male) adult Beagle dogs. Methods: Medetomidine (450 μg m−2) was administered intramuscularly, followed by either S-ketamine (2 mg kg−1) or RS-ketamine (4 mg kg−1) 20 minutes later, both administered intravenously. Blood samples were collected before medetomidine administration and at multiple time points 1–900 minutes following the ketamine administration. Plasma samples were analysed using liquid chromatography–tandem mass spectrometry. Heart rate, respiratory rate, noninvasive blood pressure, haemoglobin saturation with oxygen and body temperature were measured at baseline, before ketamine administration, and 1, 2, 5, 10, 15, 20 and 30 minutes after ketamine administration. All cardiovascular variables, blood glucose, haemoglobin and lactate concentrations were analysed using different linear mixed effects models; the significance was set at $p < 0.05$. Results: S-ketamine showed a two-compartment kinetic profile; no statistically significant differences were observed between its concentrations or in the calculated pharmacokinetic parameters following S- or RS-ketamine. When the racemic mixture was administered, no differences were detected between R- and S-ketamine concentrations, but the area under the curve (AUC) for R-norketamine was significantly lower than that for S-norketamine. Clinically relevant physiologic variables did not show statistically significant differences following the administration of the racemic mixture or of S-ketamine alone. Conclusions and clinical relevance: This study performed in dogs showed that RS-ketamine and S-ketamine combined with medetomidine showed enantioselective pharmacokinetics as S- and R-norketamine AUCs were different, but S-ketamine levels were identical.

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RESEARCH PAPER

Pharmacokinetics of S-ketamine and R-ketamine and their active metabolites after racemic ketamine or S-ketamine intravenous administration in dogs sedated with medetomidine

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Abstract

Objective To assess the differences in the pharmacokinetic profiles of S-ketamine, R-ketamine and their metabolites, S-norketamine and R-norketamine, and to measure relevant physiologic variables after intravenous administration of racemic (RS) ketamine or S-ketamine alone in Beagle dogs sedated with medetomidine.

Study design Experimental, blinded and randomized crossover study.

Animals A total of six (three female and three male) adult Beagle dogs.

Methods Medetomidine (450 μg m^{-2}) was administered intramuscularly, followed by either S-ketamine (2 mg kg^{-1}) or RS-ketamine (4 mg kg^{-1}) 20 minutes later, both administered intravenously. Blood samples were collected before medetomidine administration and at multiple time points 1−900 minutes following the ketamine administration. Plasma samples were analysed using liquid chromatography−tandem mass spectrometry. Heart rate, respiratory rate, noninvasive blood pressure, haemoglobin saturation with oxygen and body temperature were measured at baseline, before ketamine administration, and 1, 2, 5, 10, 15, 20 and 30 minutes after ketamine administration. All cardiovascular variables, blood glucose, haemoglobin and lactate concentrations were analysed using different linear mixed effects models; the significance was set at $p < 0.05$.

Results S-ketamine showed a two-compartment kinetic profile; no statistically significant differences were observed between its concentrations or in the calculated pharmacokinetic parameters following S- or RS-ketamine. When the racemic mixture was administered, no differences were detected between R- and S-ketamine concentrations, but the area under the curve (AUC) for R-norketamine was significantly lower than that for S-norketamine. Clinically relevant physiologic variables did not show statistically significant differences following the administration of the racemic mixture or of S-ketamine alone.

Conclusions and clinical relevance This study performed in dogs showed that RS-ketamine and S-ketamine combined with medetomidine showed enantioselective pharmacokinetics as S- and R-norketamine AUCs were different, but S-ketamine levels were identical.

Keywords canine, medetomidine, pharmacokinetics, S-ketamine, S-norketamine.

Introduction

Ketamine is a dissociative anaesthetic, widely used in human and veterinary anaesthesia. It is a racemic mixture of two optical isomers, R- and S-ketamine, which have different pharmacological effects (Bergman 1999). S-ketamine is available for dogs in some European countries. In humans, the relative potency of S-ketamine is twice that of the racemic (RS) ketamine, and the loss of response to verbal commands is seen at half the dose of S-ketamine compared with RS-ketamine (Ihmsen et al. 2001). In dogs and ponies, the clearance of S-ketamine administered alone is higher than that of S-ketamine or R-ketamine administered in the racemic form (Ihmsen et al.

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2001; Duque et al. 2008; Larenza et al. 2009). This explains the faster recovery seen in patients anaesthetized with the S-enantiomer alone (Ihmsen et al. 2001; Duque et al. 2008).

Clinically relevant physiologic functions are usually well maintained with ketamine when in combination with moderate doses of α_2-adrenoceptor agonists. Studies in ponies and horses (Filzek et al. 2003; Larenza et al. 2007), however, have shown some differences in the cardiopulmonary effects related to the stereoselectivity of ketamine.

The combination of RS-ketamine with α_2-adrenoceptor agonists has been widely used in dogs to induce sedation and anaesthesia (Ueyema et al. 2008). This drug combination produces an adequate quality and duration of sedation and anaesthesia for minor medical and surgical procedures. α_2-Adrenoceptor agonists alter the metabolism of other co-administered drugs, such as opioids and ketamine *in vitro*, mainly via an interaction with cytochrome P (CYP) enzymes (Kharasch et al. 1991; Sandbaumhüter et al. 2015). This interaction can influence the intensity and duration of the effects of ketamine.

The aims of this study were to obtain the pharmacokinetic profiles of S-ketamine and R-ketamine, and their major metabolites, S-norketamine and R-norketamine, in healthy Beagles sedated with intramuscular (IM) medetomidine after intravenous (IV) administration of RS-ketamine or S-ketamine. Clinically relevant physiologic variables were also compared.

We hypothesized that the pharmacokinetics of ketamine's enantiomers and its metabolites, when combined with medetomidine, were not stereoselective in dogs administered RS-ketamine or the S-isoform alone.

Material and methods

Animals

The trial was approved by the committee for Animal Experimentation of XXX 67/2011. A total of six healthy adult Beagle dogs, three females and three males (non-castrated), aged 21 ± 11 [mean ± standard deviation (SD)] months of age and weighing 15.0 ± 1.1 (mean ± SD) kg, were used in the study. A *post hoc* power calculation on the pharmacokinetic data was used to verify the adequacy of the number of dogs included, and the power was 99.5% for six dogs.

Complete blood cell count and blood chemistry were assessed 2 days before the experiment. The dogs were fasted overnight, but always had free access to water.

Drug administration and monitoring

This was a blinded, randomized crossover trial with a 3 week washout period between treatments. A random order generator (GraphPad Software, CA, USA) determined treatment allocation. The study was performed following good clinical practice guidelines (Flecknell 1993).

Following standard aseptic preparation, two peripheral venous catheters (Surflo IV Catheter 22 gauge, Terumo, Belgium) were inserted into both the cephalic veins of each dog, one catheter for drug administration and the other as a reserve. An additional catheter was placed in the jugular vein (16 gauge, 16 cm long, Blue Flex Tip Catheter, Arrow International, Teleflex Medical GmbH, Switzerland) following infiltration of lidocaine (1 mL; Lidocain 2%, Streuli, Switzerland). This catheter was used for central venous blood sampling. The catheter for drug administration was attached to a lactated Ringer infusion (Ringer-Lactat Fresenius; Fresenius Kabi AG, Switzerland) at a rate of 5 mL kg^{-1} $hour^{-1}$ for 30 minutes prior to anaesthesia induction. The dogs were sedated with IM medetomidine administered at a dose rate of 450 μg m^{-2} (approximately 17 μg kg^{-1} in a 15 kg dog, Dorbene, Fort Dodge, Italy). After 20 minutes, S-ketamine 2 mg kg^{-1} (Keta-S; Dr. E. Graeub AG, Switzerland) (S-KET treatment) or RS-ketamine 4 mg kg^{-1} (Ketasol-100; Dr. E. Graeub AG) (RS-KET treatment) were rapidly administered IV over 1−2 seconds.

Once anaesthesia was induced (identified by loss of laryngeal reflex), the dogs were intubated and allowed to breath room air (fraction of inspired oxygen = 0.21). A multiparameter monitor (BN 850, GE Medical Systems, Anandic Medical Systems AG, Switzerland) was used to monitor anaesthesia. The following measurements were taken: heart rate (HR) from an electrocardiogram, respiratory rate (f_R), indirect arterial blood pressures [systolic (SAP), mean (MAP) and diastolic (DAP)], haemoglobin (Hb) saturation with oxygen in % (SpO_2), end-tidal carbon dioxide ($P_E'CO_2$) and body temperature (T°). These variables were recorded at baseline [before medetomidine administration = time point (T-22)], at T-1 (20 minutes after medetomidine injection and prior to ketamine administration), and 1, 2, 5, 10, 15, 20 and 30 minutes after the ketamine administration. Before ketamine administration, and 5 and 30 minutes after ketamine injection, blood glucose (Contour, Bayer AG Heathcare, Switzerland), lactate (Accutrend, Roche Diagnostics, Switzerland) and Hb concentration (Hemocue Hb201+, Baumann Medical AG, Switzerland) were measured.

The peripheral venous catheters were removed 30 minutes after drug administration. The dogs were given 4 mg kg^{-1} IV carprofen (Rimadyl ad us. Vet, Pfizer AG, Switzerland) 4 hours after drug administration. The dogs were observed by veterinarians and offered a commercial diet once they were fully awake. During and after anaesthesia, the dogs' body temperatures were maintained at 37.0−38.5 °C using warm water blankets (Hico-Aquatherm 660, Nufer Medical, Switzerland), a heat and moisture exchanger (HMEF1000, Anandic Medical

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Systems AG) and a forced air-patient warming system (Bair Hugger Model 505, Carbamed, Switzerland) as needed. After taking the last blood sample, the jugular catheter was removed.

Sample collection

A blood sample was taken for determination of plasma drug concentration 2 minutes before medetomidine administration (T-22) and 20 minutes after (T-2), prior to anaesthesia induction. Blood samples were also collected at anaesthesia induction (T0), 1, 2, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, 450, 540, 630, 720, 810 and 900 minutes after ketamine administration. Samples for determination of plasma drug levels were collected from the central venous catheter and put into labelled heparinized tubes (BD 3.5 mL Vacutainer, Becton Dickinson, Belgium). A total of 4 mL of blood were drawn before each sample and were reinjected immediately after, and the catheter was then flushed with 5 mL of saline (B-Braun, Melsungen, Germany). The effective time of sampling was recorded for each sample. Immediately after collection, the samples were centrifuged at 4 °C and 3000 ***g*** for 10 minutes (Sarstedt LC 1-K, Germany). The plasma was stored at −80 °C in suitable tubes (Nunc 1.8 mL SI Cryotube vials; Nunc A/S, Denmark) until analysis.

Plasma drug analysis

The plasma samples were analysed using the liquid chromatography tandem mass spectrometry (LC-MS/MS) method, previously described by Romagnoli et al. (2017). Briefly, after the addition of labelled internal standards (Ketamine-d4 and Norketamine-d4; Sigma-Aldrich, MO, USA), 150 µL of plasma were extracted with methanol and centrifuged. The supernatant was filtered through a 0.2 µm PTFE filter (Phenomenex, CA, USA) prior to analysis.

The LC system consisted of a Waters Aquity UPLC binary pump (Waters, MA, USA), equipped with a Phenomenex Lux 3 µm Cellulose-3 (150 × 2.00 mm, 3.0 µm) column (Phenomenex). The mobile phase was a mixture of acetonitrile and an aqueous solution containing ammonium acetate 20 mM and ammonium formate 0.1% at a flow rate of 0.45 mL minutes^{-1} under programmed conditions. The LC was interfaced to a Waters Quattro Premier XE triple quadrupole mass spectrometer (Waters), operating in positive electrospray ionization, and two specific transitions were observed for each analyte (Ketamine: 238 → 125 and 179 *m/z*, Norketamine: 224 → 125 and 207 *m/z*) and for each internal standard (Ketamine-D4: 242 → 129 and 183 *m/z*, Norketamine-D4: 228 → 129 and 211 *m/z*).

The analytical method was validated in accordance with EMEA/CHMP/EWP/192217/2009 guidelines at the beginning of the experiment. Linearity was satisfactory ($R^2 > 0.99$) over a range extending from 15 to 15,000 ng mL^{-1} for both S-ketamine and R-ketamine, and from 15 to 3000 ng mL^{-1} for the norketamine enantiomers. The lower limit of quantification was 15 ng mL^{-1} for all target compounds; interday and intraday accuracy and precision were both below 10% for all the analytes.

Pharmacokinetics and statistical analysis

The aim of the statistical analysis was to detect potential differences in the repeated measurements of the cardiopulmonary variables, Hb, lactate and glucose concentrations between the two treatments. For each of the outcome variables, different linear mixed effects models were run. In all models, dog was included as a random intercept to account for potential clustering within animals. In contrast, in the different models, time was included either as a fixed effect, with or without an interaction term with treatment, or as a random slope. The treatments were included as a fixed effect and omitted in the null model. Model selection was based on Akaike's information criterion (AIC) and on likelihood ratio tests which provided the *p* values.

The analysis was carried out using R software (R Core Team 2018) and the packages: nlme (Pinheiro et al. 2018) and lmtest (Zeileis & Hothorn 2002). Based on the assumption of missing at random, the missing values were provided using the package missForest (Stekhoven 2013). All cardiopulmonary variables, Hb, lactate and glucose concentrations are reported as mean ± SD.

The R-ketamine and S-ketamine concentration *versus* time curves were analysed for each individual by XY plot using WinNonlin 6.3 (Pharsight Corporation, CA, USA).

The plasma drug concentrations obtained after IV administration were fitted using the following equation:

$$C(t) = A\ e^{-at} + B\ e^{-\beta t}.$$

All pharmacokinetic parameters are reported as mean ± SD and were determined using WinNonlin 6.3 (Pharsight Corporation). The individual plasma concentration *versus* time curves were fitted, and the best compartment model was determined by application of the AIC (Yamaoka et al. 1978).

The following pharmacokinetic parameters were calculated for each dog for the ketamine enantiomers: area under the curve to infinity ($AUC_{0\rightarrow\infty}$), half-life of the distribution phase ($T_{1/2dis}$), half-life of the elimination phase ($T_{1/2el}$), rate constants of the elimination phase (K_{el}), mean residence time (MRT), total body clearance (Cl_B), volume of distribution of the central compartment (V_c), peak concentration (C_{max}) and time of peak concentration (T_{max}). For S-norketamine and R-norketamine, non-compartmental analysis was used to determine

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the AUC $_{0\rightarrow\infty}$, C_{max} and T_{max} of metabolite concentration. The Wilcoxon signed-rank test was used to detect differences between the treatments concerning the pharmacokinetic parameters with a significance level of $p < 0.05$.

Results

All the dogs enrolled in the study also finished the study and recovered without any complications. The dogs came from the pool of experimental dogs of the Vetsuisse Faculty of the University of Zürich, where they returned 24 hours after the end of the study.

Sedation was considered to be profound following medetomidine administration, and S- and RS-ketamine were injected according to the scheduled times. Intubation was judged to be easy in four dogs in the RS-KET treatment and two dogs in the S-KET treatment, and less easy but possible in the remaining dogs. In the RS-KET treatment, one dog showed several episodes of muscle shaking 3.5 minutes following drug administration. All dogs recovered well from the anaesthesia and were standing within 37.9 ± 16.9 minutes and 42.9 ± 19.6 minutes in S-KET and RS-KET treatments, respectively. No significant difference was detected between treatments.

The cardiovascular variables are summarized in Table 1. The SpO_2 ranged between 98% and 100% in all dogs. Some cardiopulmonary measurements were only possible when the dogs were unconscious and tolerated the endotracheal tube (e.g., $P_{E'}CO_2$) or other measurement devices.

No significant treatment effect was detected in the following variables: HR ($p = 0.068$), f_R ($p = 0.388$), SAP ($p = 0.465$), DAP ($p = 0.260$), MAP ($p = 0.355$), T° ($p = 0.317$), SpO_2 ($p = 0.967$), Hb ($p = 0.09$), lactate ($p = 0.230$) and glucose ($p = 0.185$).

Plasma drug concentrations

Due to technical problems with the drug assay, the concentrations of R- and S-enantiomers from the racemic mixture could not be determined in one dog.

The plasma drug concentrations of both the ketamine and norketamine enantiomers were plotted against the time points for both RS-ketamine and S-ketamine administration (Figs. 1 & 2). No statistically significant differences were detected between the concentrations of S-ketamine alone, and R-ketamine and S-ketamine after administration of the racemic mixture, at any time. Neither R-ketamine nor R-norketamine were detected after the administration of S-ketamine.

Pharmacokinetic parameters

The S-ketamine plasma concentration following the IV administration of a bolus of RS-ketamine or S-ketamine alone

Table 1 Mean ± standard deviation values of heart rate (HR), systolic (SAP), diastolic (DAP), mean arterial pressure (MAP), respiratory rate (f_R), end-tidal carbon dioxide ($P_{E'}CO_2$), glucose, lactate plasma concentrations and haemoglobin (Hb) in six Beagle dogs. The dogs were sedated with medetomidine (450 μg m^{-2}) and given a bolus of S-ketamine [S-KET (2 mg kg^{-1})] or racemic ketamine [RS-KET (4 mg kg^{-1})] 20 minutes after medetomidine administration (T0)

Time (minutes)	Treatment	Baseline	0	1	2	5	10	15	20	30
HR (beats $minute^{-1}$)	S-KET	103 ± 16	48 ± 3	82 ± 16	82 ± 21	84 ± 8	75 ± 19	68 ± 21	60 ± 7	58 ± 18
	RS-KET	108 ± 19	53 ± 9	100 ± 9	108 ± 23	86 ± 9	74 ± 7	60 ± 7	54 ± 5	54 ± 5
SAP (mmHg)	S-KET			197 ± 0	197 ± 40	151 ± 25	133 ± 36	145 ± 46	137 ± 35	147 ± 5
	RS-KET				154 ± 45	162 ± 38	153 ± 26	159 ± 27	158 ± 21	141 ± 13
DAP (mmHg)	S-KET			137 ± 0	118 ± 42	98 ± 30	84 ± 23	81 ± 23	86 ± 27	10 ± 8
	RS-KET				97 ± 43	96 ± 24	96 ± 33	96 ± 24	99 ± 23	82 ± 21
MAP (mmHg)	S-KET			161 ± 0	144 ± 40	120 ± 29	102 ± 29	107 ± 33	107 ± 29	121 ± 5
	RS-KET				116 ± 39	126 ± 34	117 ± 30	120 ± 23	122 ± 20	105 ± 17
f_R (breaths $minute^{-1}$)	S-KET	28 ± 5	19 ± 5	8 ± 7	9 ± 6	10 ± 6	17 ± 7	22 ± 7	23 ± 4	24 ± 9
	RS-KET	25 ± 7	20 ± 6	13 ± 12	8 ± 8	7 ± 4	12 ± 5	23 ± 20	23 ± 8	28 ± 6
$P_{E'}CO_2$ (kPa)	S-KET				4.4 ± 1.3	6.3 ± 0.3	5.6 ± 1.9			
	RS-KET				5.8 ± 1.5	7.0 ± 0.4	6.6 ± 0.6	6.1 ± 0.0		
$P_{E'}CO_2$ (mmHg)	S-KET				33.0 ± 9.9	47.6 ± 2.3	42.0 ± 14.2			
	RS-KET				43.3 ± 11.0	52.2 ± 3.0	49.3 ± 4.2	46.0 ± 0.0		
Glucose (mmol L^{-1})	S-KET		4.2 ± 0.4			4.5 ± 0.7				4.7 ±1.0
	RS-KET		4.6 ± 0.8			4.3 ± 0.7				4.6 ± 0.7
Lactate (mmol L^{-1})	S-KET		5.4 ± 3.7			2.3 ± 0.5				2.0 ± 1.1
	RS-KET		2.4 ± 1.1			1.8 ± 0.9				2.5 ± 0.3
Hb (g dL^{-1})	S-KET		14.4 ± 1.2			13.4 ± 1.5				11.9* ± 1.2
	RS-KET		13.6 ± 1.9			13.8 ± 1.0				13.7 ± 0.9

* Significant difference between the treatments, $p < 0.05$.

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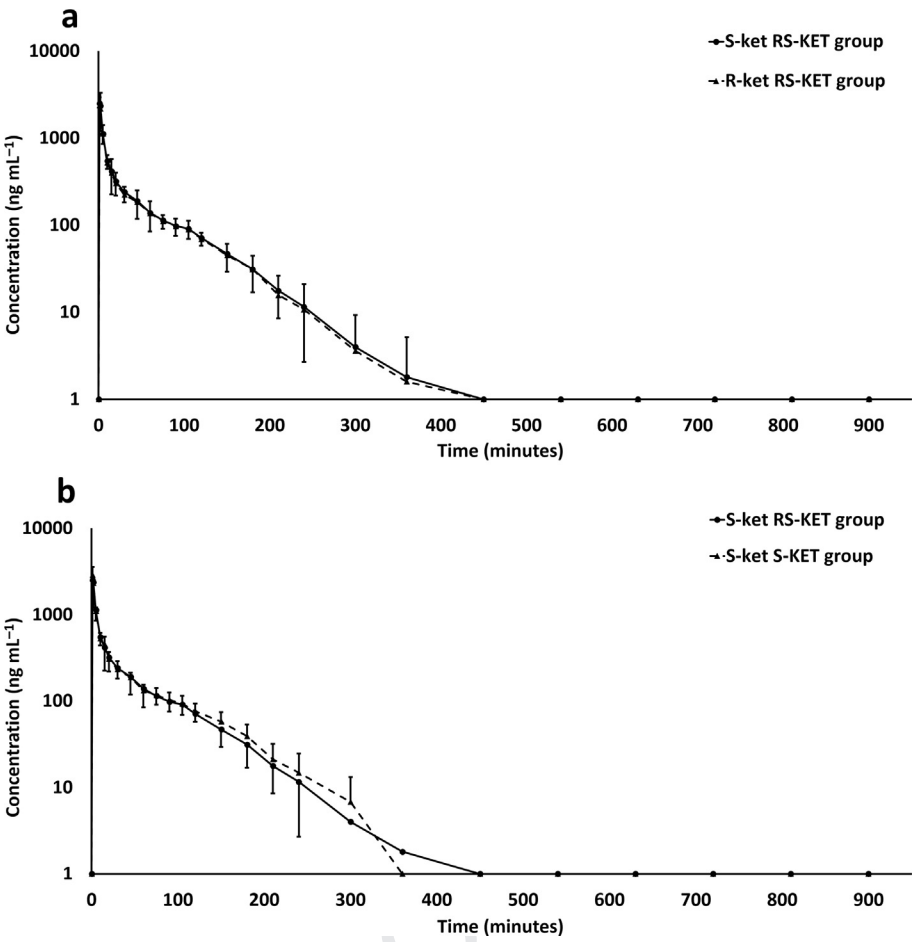

Figure 1 Mean plasma concentrations (error bars represent standard deviation) of R-ketamine (R-ket) and S-ketamine (S-ket) for the RS-KET treatment graph (a) and S-ketamine (S-ket) for the S-KET treatment graph (b) after the administration of racemic ketamine 4 mg kg^{-1} or S-ketamine 2 mg kg^{-1}, respectively, to five dogs sedated with medetomidine (450 μg m^{-2}).

was best described using a two-compartment model. The pharmacokinetic parameters are summarized in Table 2. No statistically significant differences were observed in S-enantiomer concentrations between treatments. Furthermore, there was no significant difference between two enantiomers in the RS-KET treatment. The $AUC_{0\rightarrow\infty}$ for S-norketamine, after both RS-ketamine and S-ketamine administration, was significantly higher than that for R-norketamine measured after injection of the racemic mixture.

Discussion

In this study, following IM administration of medetomidine, the pharmacokinetic parameters of S-ketamine after a single IV injection of the racemic drug or the S-enantiomer alone did not differ significantly from one another. In addition, the pharmacokinetic results for the R-isomer did not differ significantly from those of S-ketamine after RS-ketamine administration either.

The cardiovascular and respiratory effects observed following medetomidine and ketamine administration are in accordance with those previously reported for protocols including ketamine and medetomidine (Ko et al. 2001; Enouri et al. 2008). α_2-Adrenoceptor agonist administration commonly causes bradycardia, as observed in our study. This results from both an increase in systemic vascular resistance and a decrease in sympathetic tone (Pypendop & Verstegen 1998). In the present study, it was not possible to evaluate the noninvasive blood pressure before RS-ketamine or S-ketamine administration due to the temperament of the dogs. However, the administration of both the RS-ketamine and S-ketamine induced an increase in HR, as previously reported in dogs by Enouri et al. (2008). In the present study, the cardiovascular effects of the two different anaesthetic protocols

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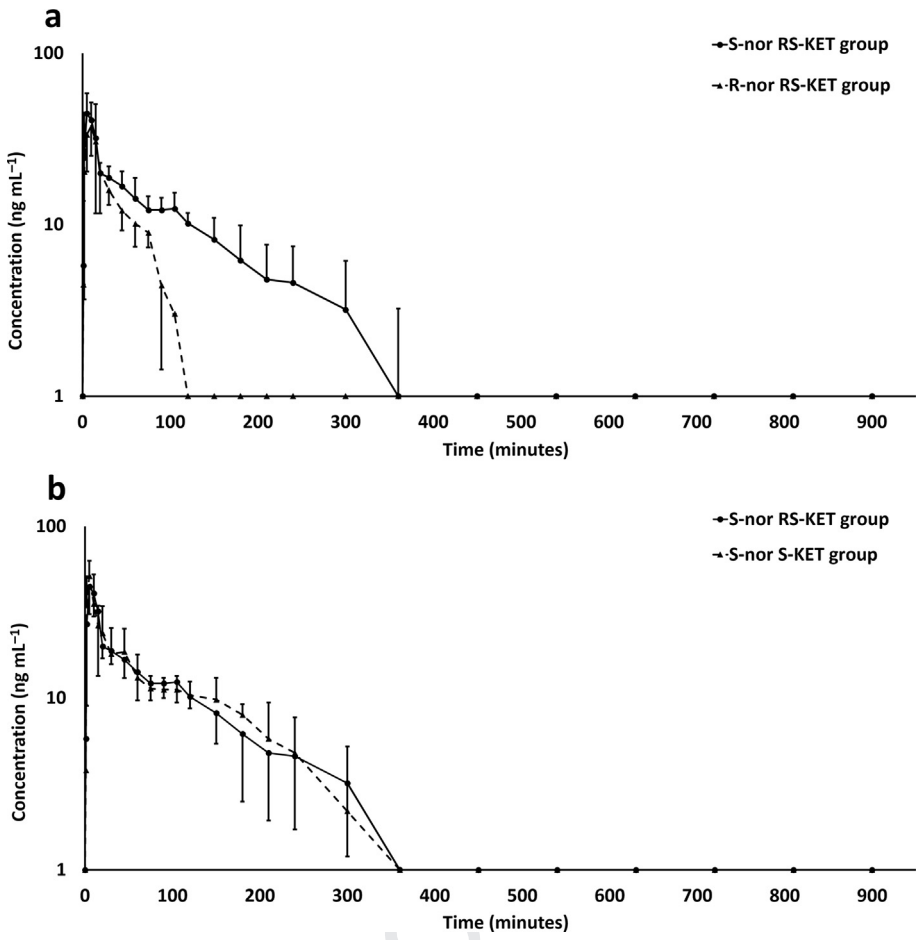

Figure 2 Mean plasma concentrations (error bars represent standard deviation) of R-norketamine (R-nor) and S-norketamine (S-nor) in the RS-KET treatment graph (a) and S-norketamine (S-nor) in the S-KET treatment graph (b) after the administration of racemic ketamine 4 mg kg^{-1} or S-ketamine 2 mg kg^{-1}, respectively, to five dogs sedated with medetomidine (450 µg m^{-2}).

were comparable, as were the recovery times. In human volunteers sedated with midazolam, S-ketamine provided faster recovery than RS-ketamine administered at twice the dose of S-ketamine alone (Doenicke et al. 1992).

In the present study, concentration of S-norketamine was significantly more than that of R-norketamine in plasma. These findings disagree with those reported by Sandbaumhüter et al. (2016); they did not find any statistically significant differences between the two enantiomers in Beagle dogs. Moreover, lower overall S-norketamine and R-norketamine concentrations were observed in the dogs included in the present study than dogs anaesthetized with sevoflurane (Romagnoli et al. 2017). Mechanical ventilation and sevoflurane may influence cardiovascular function and thus drug disposition and elimination (Romagnoli et al. 2017). In the present study, all the dogs were sedated with medetomidine, which also has an influence on the ketamine metabolism (Kharasch et al. 1992; Sandbaumhüter et al. 2016). Such variation might be related to a reduction of the cardiac output (CO). In a previous study, Pypendop & Verstegen (1998) reported that medetomidine administered to healthy dogs significantly reduced CO and may thereby reduce hepatic perfusion. Conversely, Lawrence et al. (1996) reported that dexmedetomidine, the active enantiomer of medetomidine, decreased renal blood flow by 25% but did not affect liver blood flow. Furthermore, Restitutti et al. (2013), observed that dexmedetomidine induced changes in the blood flow of the abdominal organs, especially in the kidneys. In the study reported by Sandbaumhüter et al. (2016), no significant differences were detected in the metabolic profile of ketamine of urine when dogs were administered either sevoflurane or medetomidine. Therefore, it seemed likely that both medetomidine and sevoflurane could decrease ketamine elimination. The hepatic metabolism of both ketamine and medetomidine is

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Table 2 Mean values ± standard deviation of the pharmacokinetic parameters of ketamine and norketamine enantiomers in plasma samples obtained from five dogs sedated with medetomidine and an intravenous bolus of S-ketamine (S-KET) or racemic ketamine (RS-KET) (refer to Table 1 for drugs doses administered)

Treatment	S-KET	RS-KET	
Compound	S-ketamine	S-ketamine	R-ketamine
$AUC_{0\rightarrow\infty}$ (μg minutes mL^{-1})	37.21 ± 5.28	36.83 ± 13.00	35.58 ± 12.15
$T_{1/2dis}$ (minutes)	2.47 ± 1.09	2.44 ± 1.39	2.52 ± 1.35
$T_{1/2el}$ (minutes)	43.77 ± 20.12	46.02 ± 18.51	46.74 ± 18.08
K_{el} (1 $minute^{-1}$)	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01
MRT (minutes)	45.00 ± 20.23	45.25 ± 16.00	46.39 ± 15.38
Cl_B (mL $minute^{-1}$ kg^{-1})	54.62 ± 7.73	59.82 ± 20.94	61.78 ± 21.76
V_c (L kg^{-1})	1.79 ± 0.69	1.94 ± 0.52	2.03 ± 0.57
	S-norketamine	S-norketamine	R-norketamine
$AUC_{0\rightarrow\infty}$ (μg*minutes mL^{-1})	3.27 ± 0.94*	3.29 ± 0.86†	1.45 ± 0.25*†'
C_{max} (μg mL^{-1})	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
T_{max} (minutes)	6.00 ± 2.24	7.40 ± 5.13	8.40 ± 6.14

$AUC_{0-\infty}$, Area under the curve to infinity; $T_{½dis}$, half-life of the distribution phase; $T_{½el}$, half-life of the elimination phase; K_{el}, rate constants of the elimination phase; MRT, mean residence time; Cl_B, body clearance; V_c, volume of distribution for the central compartment; C_{max}, peak concentration; T_{max}, time of peak concentration. *Significant difference between the treatments, †significant difference within the groups, $p < 0.05$.

catalysed by CYP3A4 and orthologs of CYP2C9 enzymes (Capponi et al. 2009; Schmitz et al. 2010), and they have also shown an elevated affinity for medetomidine in dogs (Duhamel et al. 2010). α_2-Adrenoceptor agonists inhibit CYP450 by means of their imidazole ring which binds to the haem iron of CYP (Sandbaumhüter et al. 2015) and they, therefore, slow the metabolism of drugs which use the same enzymatic pathway as demonstrated *in vitro*.

In this study, neither the V_c nor Cl_B of S-ketamine and R-ketamine differed significantly from one another in dogs administered RS-ketamine; nor did these parameters differ when the values of S-ketamine were compared to RS-ketamine. Our findings were in accordance with those previously reported in dogs anaesthetized with sevoflurane and given RS-ketamine or the S-isomer (Romagnoli et al. 2017). They suggest the absence of stereoselectivity in the distribution and clearance of ketamine enantiomers in dogs sedated with medetomidine. Similar results have previously been obtained in ponies receiving RS-ketamine or S-ketamine and anaesthetized with isoflurane or sedated with xylazine (Larenza et al. 2007; 2008).

Lower $AUC_{0\rightarrow\infty}$ and C_{max} for R-norketamine compared to S-norketamine have already been reported in ponies anaesthetized with isoflurane or xylazine (Larenza et al. 2007, 2008). Some authors have hypothesized the existence of differences in protein binding between the two enantiomers which could have influenced their renal clearance (Larenza et al. 2007). A previous *in vitro* study has demonstrated that the co-administration of medetomidine and RS-ketamine produced a stronger inhibition of the formation of R-norketamine than S-norketamine (Sandbaumhüter et al. 2015). In the present study, we hypothesized that medetomidine inhibited RS-ketamine demethylation to R-norketamine in preference for S-norketamine. Since norketamine metabolites were not evaluated, the preference of pharmacologically inactive R-norketamine over the active S-norketamine hydroxylation cannot be excluded. However, the pharmacokinetics of 6-hydroxynorketamine and dehydronorketamine enantiomers have already been determined in dogs sedated with medetomidine (Sandbaumhüter et al. 2016). In that study, a higher C_{max} of (2R,6R)-6-hydroxynorketamine compared with (2S,6S)-6-hydroxynorketamine was observed after administration of the racemic mixture; similar results were not found for 5,6-dehydronorketamine. In the study of Sandbaumhüter et al. (2016), there were no differences between R- and S-norketamine pharmacokinetics. However, in the present study, inhibition of the formation of the R-isoform over the S-isoform of norketamine was observed following medetomidine administration. In canine clinical practice, the pharmacological effect of ketamine metabolites, particularly S-norketamine, is still unclear. However, an analgesic effect similar to that of RS-ketamine was reported for this metabolite in a rodent model (Holtman et al. 2008). In the present study, no analgesic evaluations were performed in Beagle dogs.

Both dexmedetomidine and levomedetomidine are potent *in vitro* inhibitors of the N-demethylation of S- and R-ketamine to norketamine (Kharasch et al. 1992). Hence, racemic medetomidine was expected by the author of the present study to be a more potent inhibitor than dexmedetomidine alone. Additional studies are needed to evaluate the effects of

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dexmedetomidine on ketamine and norketamine disposition, and pharmacokinetics with respect to medetomidine.

This study has some limitations; there was a high intra- and inter-individual variability of cardiorespiratory variables. In addition, some dogs did not tolerate monitoring devices being attached while awake; thus, some measurements before and after sedation and anaesthesia were not performed. As the variability was higher than expected, the study was underpowered with regards to cardiorespiratory measurements and, therefore, could not detect whether there were any differences between treatments. Moreover, the exclusion of one dog from the statistical analysis could result in a low statistical power, thereby increasing the risk of beta error.

Conclusions

This study confirmed that the distribution and clearance of ketamine enantiomers, when combined with medetomidine, were not stereoselective in dogs administered RS-ketamine or the S-isoform alone. However, the metabolism of ketamine was inhibited, as demonstrated by low norketamine concentrations with R-norketamine being the most affected. Despite these differences in metabolite disposition, no significant differences between the two treatments were observed regarding the cardiopulmonary variables studied.

Uncited reference

Q8 Kharasch and Labroo, 1992.

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Authors' contributions

NR: data acquisition, analysis and interpretation of data, conception of study design, drafted and revised the paper, approved the final version. RB: data acquisition, data interpretation, revised the paper, approved the final version. RNB and APK: data acquisition, data interpretation, revised the paper. AB, PR and SH: analysis and interpretation of data, drafted the paper.

Conflict of interest statement

The authors declare no conflict of interest.

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